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ORTHO-ALKOXYPHENOL LEUKOTRIENE B_4 RECEPTOR ANTAGONISTS: EFFECT OF A CHROMAN CARBOXYLIC ACID.

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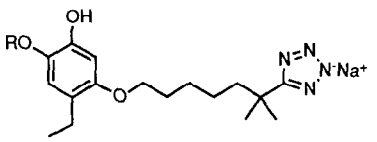
Abstract: Several ortho-alkoxyphenols containing a chroman carboxylic acid sidechain have been prepared as antagonists of leukotriene B_4 receptors. These antagonists were compared to their parent alkoxyphenols containing the tetrazole acid sidechain. These chroman containing antagonists retained their binding potency for human neutrophil receptors; however, showed enhanced potency against guinea pig receptors in both *in vitro* and *in vivo* systems.

We have reported that ortho-alkoxyphenols of general structure **1** are potent leukotriene B_4 receptor antagonists.[1] Relative to the ortho-hydroxyacetophenones, these ortho-alkoxyphenol antagonists showed enhanced activity *in vitro* in human neutrophil and guinea pig lung receptor binding assays and *in vivo* in a guinea pig model of LTB_4-induced airway obstruction. Structurally, the ortho-alkoxyphenol moiety demonstrated that the acyl group of the ortho-hydroxyacetophenone antagonist (LY255283) is not critical for receptor-antagonist recognition, and that the receptor can accommodate relatively modest size ether substituents attached ortho to the phenol.



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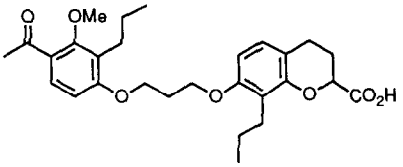


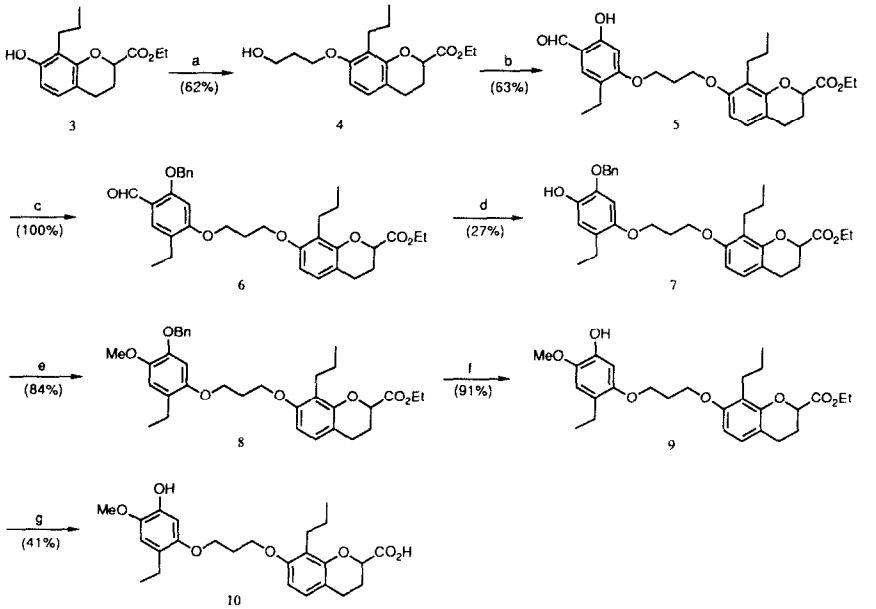
2 (SC-41930)

In an effort to further optimize the potency of the ortho-alkoxyphenol class of antagonists, we directed our attention to the gem-dimethyltetrazole acid portion of the molecule. From the available SAR on the ortho-hydroxyacetophenones, it was clear that the gem-dimethyl substitution adjacent to the tetrazole acid was critical for potency.[2] This finding indicated that at the receptor site a lipophilic binding region existed near the acid binding site. Thus, an acid containing an adjacent lipophilic moiety seemed critical if we wished to maintain or enhance antagonist potency. We were aware of the LTB_4 receptor antagonist SC-41930 (**2**).[3] This molecule contained a chroman carboxylic acid, an acid related to the chromone acid, a common lipophilic acid unit found in many

leukotriene antagonist series.[4] We proceeded to assess the compatability of the chroman carboxylic acid with our ortho alkoxyphenol core structure.

Based on the potency of the gem-dimethyltetrazole analogues, the ortho-methoxy- and ortho-ethoxyphenol chroman acids were selected as our initial candidates for evaluation.[1] We prepared each of these antagonists via synthetic routes which differed by the sequence in which the chroman unit was attached to the phenolic portion of the molecule. Our initial approach to these systems is demonstrated by the preparation of the ortho-methoxyphenol chroman acid analogue **10** (see Scheme 1). In this approach, we envisioned compound **7** as a versatile intermediate for the preparation of several analogues. Intermediate **7** was prepared by alkylation of the phenol of chroman ester **3**[3,5] with allylbromide. Hydroboration-oxidation provided the primary alcohol **4** which was used in the chemoselective phenol alkylation of 2,4-dihydroxy-5-ethyl benzaldehyde as we described previously.[1] The remaining phenol was protected as its benzyl ether. Next ,Baeyer-Villiger oxidation of the aromatic aldehyde did provide the key intermediate **7**; however, this oxidation did not proceed as cleanly as had been observed in previous cases where the gem-dimethylnitrile sidechain existed in place of the chroman ester sidechain.[1] Intermediate **7** was only obtained in 27% yield. Several other unidentified products were produced. The synthesis of the ortho-methoxyphenol chroman acid antagonist **10** was completed uneventfully by subsequent methylation of the free phenol, debenzylation and ester hydrolysis.

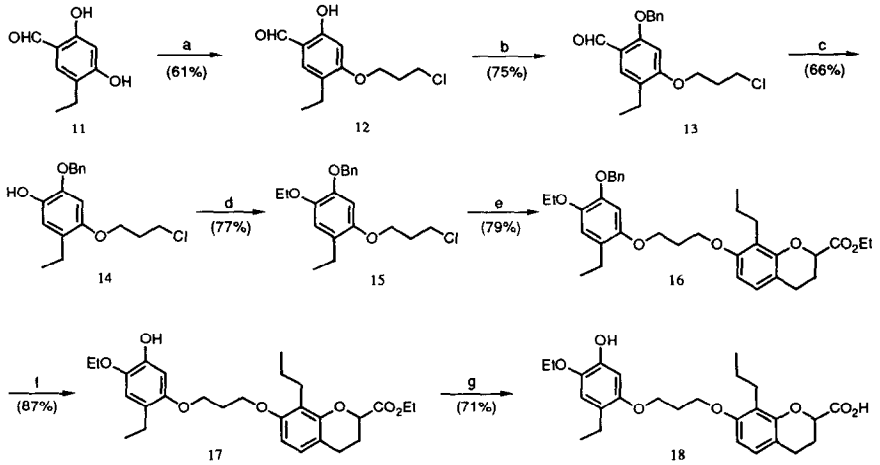
Scheme 1



Reagents: a) i) K_2CO_3, DMF, allybromide, 25°C, ii) 9-BBN, THF, iii) 30%H_2O_2, NaOH; b) DEAD, Ph_3P, THF, 2,4-dihydroxy-5-ethyl benzaldehyde; c) K_2CO_3, DMF, benzylbromide, 75°C; d) i) mCPBA, CH_2Cl_2, 25°C, ii) 2N NaOH THF; e) NaH, DMF, MeI, 25°C; f) 10% Pd/C, EtOAc, H_2(g); g) i) NaOH, H_2O, Dioxane, 25°C, ii) 5N HCl

In order to circumvent the disasterous oxidation step of the previous approach, we chose to investigate an alternative route for the synthesis of the ethoxy analogue **18** (see Scheme 2). The strategy we utilized in this alternative approach was to attach the chroman ester to the fully elaborated phenol core protected as its benzyl ether. Chemoselective alkylation of the bisphenol aldehyde **11** with 3-chloro-1-propanol appended the three carbon acid-linker-chain.[1] After the remaining phenol was protected as its benzyl ether, Baeyer-Villiger oxidation followed by hydrolysis of the resulting formate ester gave the phenol **14** in 66% purified yield. Ethylation of phenol **14** proceeded in 77% yield. Conversion of the chloride to the iodide and alkylation of the chroman phenol **3** gave the coupled product **16** in 79% yield. Debenzylation and ester hydrolysis provided the acid **18** as a white solid. This alternative approach was clearly superior because it resulted in fewer chemical steps and provided higher overall yield.

Scheme 2



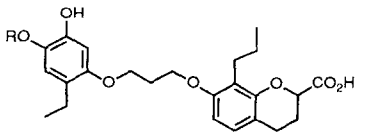
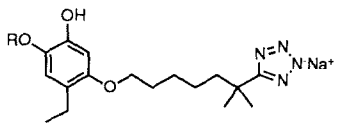
Reagents: a) DEAD, Ph_3P, THF, 3-chloro-1-propanol, 25°C; b) NaH, DMF, benzylbromide, 18-C-6; c) i) mCPBA, CH_2Cl_2, 25°C, ii) 2N NaOH, THF; d) NaH, DMF, EtI, 18-C-6, 25°C; e) i) NaI, Acetone, reflux, ii) K_2CO_3, DMF, 3; f) 10% Pd/C, EtOAc, H_2(g); g) i) 2N NaOH, H_2O, Dioxane, ii) 5N HCl.

The receptor binding affinities of the alkoxyphenol chroman acids **10** and **18** were evaluated in both human neutrophil[6] and guinea pig lung membrane[7] [^{3}H]LTB_4 radioligand binding assays (see Table 1). When the binding affinity to human neutrophil receptors for **10** and **18** were compared to their corresponding tetrazole analogues **19** and **20**, comparable binding affinities were observed. However, when the tetrazole acid was replaced by the chroman acid and the relative binding affinities against guinea pig lung membrane receptors were studied, we observed a dramatic 4- and 12-fold enhancement in potency for compounds **10** and **18**, respectively (Table 1).

In order to evaluate whether the effects we observed between structure and species in the radioligand binding studies translated into corresponding functional antagonistic effects, we evaluated the ethoxyphenol chroman **18**

in an *in vitro* assay of LTB_4-induced CD11b/CD18 integrin up-regulation in human neutrophils[8] and *in vivo* in a guinea pig model of LTB_4-induced airway obstruction (i.e. gas trapping).[9] In accordance with the results we obtained in the binding studies with human neutrophils, we saw no significant difference between the chroman acid and tetrazole acid analogues **18** and **20** in regard to their ability to antagonize the up-regulation of the CD11b/CD18 adhesion molecules (see Table 1). Also in agreement with our binding data was the relative effect observed between the chroman and tetrazole acid analogues **18** and **20** when evaluated in vivo in the guinea pig model of LTB_4-induced airway obstruction. When administered intravenously, Chroman acid **18** (ED_{50}=0.012mg/Kg) was 25-times more potent at antagonizing the effect of LTB_4-induced gas trapping in the guinea pig than was the tetrazole analogue **20** (ED_{50}=0.30mg/Kg).

Table 1. Alkoxyphenol Chroman Acid and Alkoxyphenol Tetrazole Acid Antagonists: *In Vitro* Receptor Binding and Antagonism of CD11b/CD18 Integrin Up-regulation .

Cmpd No.	Structure	Human Neutrophil Binding IC_{50} (nM)[10]	Guinea Pig Lung Membrane Binding Ki (nM)[11]	Human Neutrophil CD11b/CD18 Integrin Up-regulation IC_{50} (nM)[12]
				
	R			
10	MeO	2.9	1.93±0.72	NT[a]
18	EtO	4.2	3.51±2.42	195.0
				
	R			
19	MeO	6.0	25.1±9.2	NT[a]
20	EtO	4.8	14.2±2.9	206.0

[a]NT=not tested

From this work, it is clear that the gem-dimethyltetrazole acid unit of the ortho-alkoxyphenol class of antagonists can be substituted with an alternative lipophilic acid (chroman carboxylic acid) in order to obtain LTB_4 antagonists of equal or enhanced potency. However, the enhancement in antagonist potency seems to be relegated to their effects on guinea pig receptors and not to receptors on human neutrophils. The attachment of the chroman

acid moiety to the ortho-alkoxyphenol nucleus provides antagonists which bind equally well to both human neutrophil and guinea pig lung membrane receptors. This is in contrast to the tetrazole analogues which, as noted in our earlier study,[1] consistently showed superior potency against human neutrophil receptors as compared to guinea pig lung membrane receptors. These results provide further evidence for a species or cell specific difference in receptor structure because receptor binding and functional responses between human neutrophil and guinea pig lung were not equally effected by antagonist structural modification.

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10. For each compound, an inhibition response study was done in triplicate on cells from a single individual. For comparison of results from one individual to another, the amount of inhibition of a reference inhibitor, LY177455, was determined at 9.2μM and 0.92μM on each cell suspension. The mean percent inhibition and standard error in IC_{50} values for these compounds if studies had been done with cells from other individuals can be estimated from standard deviations of IC_{50} values obtained for compounds whose effects were measured on cells from five individuals. The average standard deviation for six LTB_4 antagonists studied in this manner was 15±4% of the mean IC_{50}.

11. Data are expressed as Mean ± SEM of values obtained from 3 to 8 experiments performed in duplicate as described in reference 7.

12. Concentration of pre-incubated antagonist (15 min @ room temp) required to provide 50% inhibition of the up-regulated CD11b/CD18 expression of human neutrophils, activated with $1x10^{-9}$M LTB_4 (30min @ 37°C). CD11b/CD18 expression was determined flow cytometrically, by measuring single cells fluorescence of specific monoclonal antibody-reacted cells (8).